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Kolattukudy

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(54) **MCPIP PROTECTION AGAINST OSTEOCLAST PRODUCTION**

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(51) **Int. Cl.**

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C12N 15/113	(2010.01)
A61K 31/713	(2006.01)
A61K 35/28	(2015.01)
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(52) **U.S. Cl.**

CPC ***C12N 15/1136*** (2013.01); ***A61K 31/713*** (2013.01); ***A61K 35/28*** (2013.01); ***C07K 16/18*** (2013.01); ***C12N 15/113*** (2013.01); *C12N 2310/11* (2013.01); *C12N 2310/14* (2013.01)

(58) **Field of Classification Search**

CPC C12N 15/1136; C12N 2310/11; A61K 35/28

See application file for complete search history.

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(57) **ABSTRACT**

Disclosed herein are methods of treating a patient at risk of developing an inflammatory joint disease. In exemplary embodiments, the method involves inhibiting MCPIP levels in a patient in need, wherein said patient in need is exhibiting pre-arthritic or pre-osteoporotic symptoms.

20 Claims, 10 Drawing Sheets

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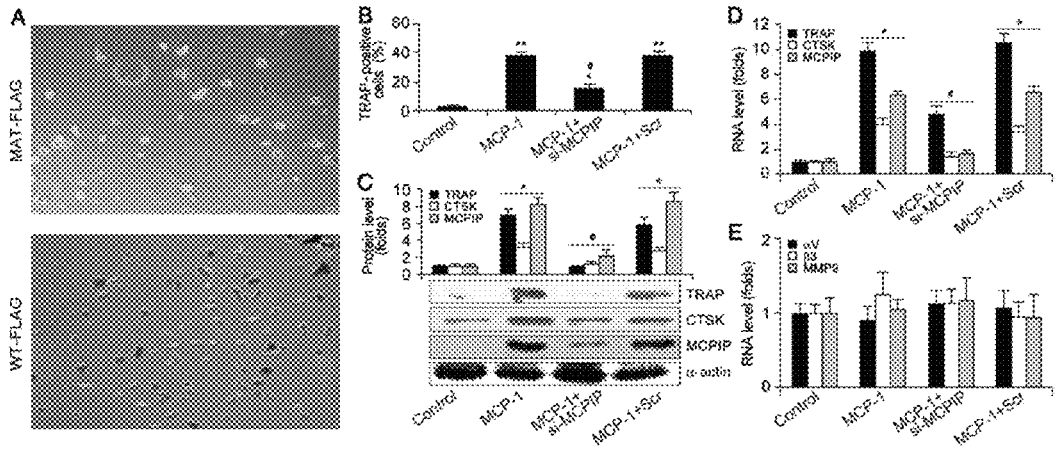
Figure 1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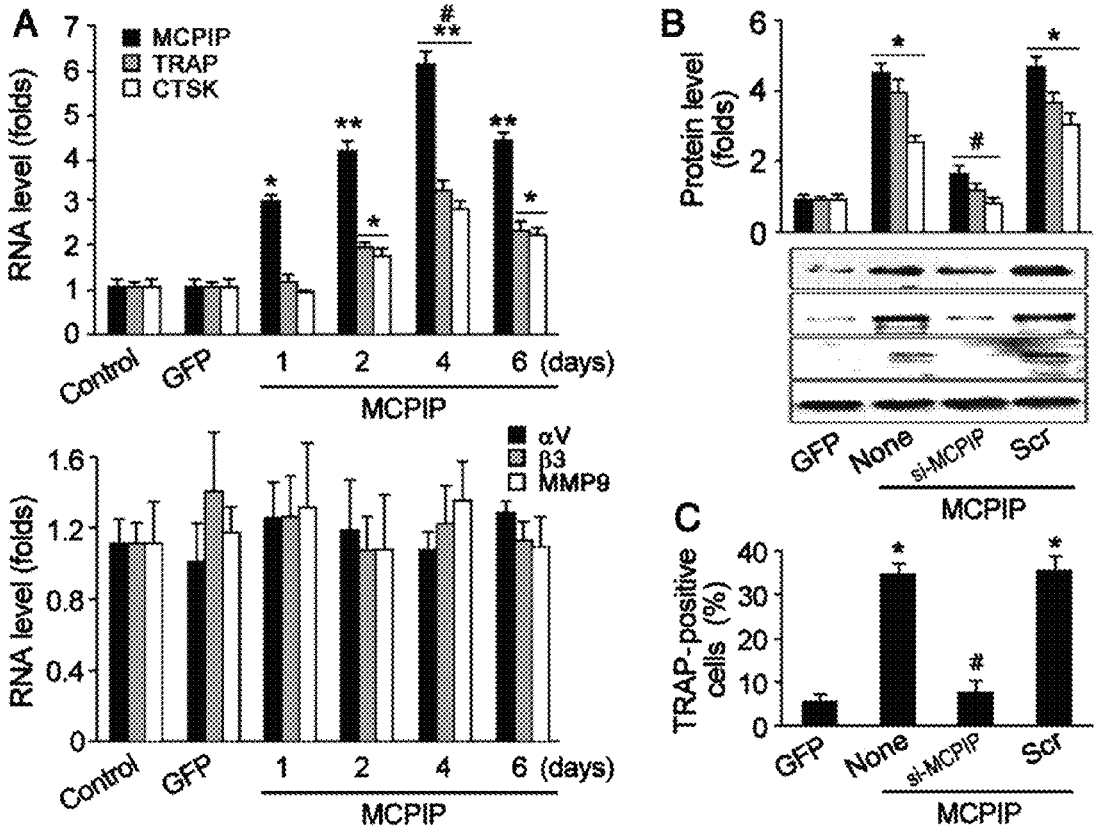
MAT-FLAG
WT-FLAG
B
TRAP-positive cells (%)
Control
MCP-1
MCP-1+ si-MCPIP
MCP-1+Scr
C
Protein level (folds)
TRAP
CTSK
MCPIP
α-actin
D
RNA level (folds)
E
αV
β3
MMP9

Figure 2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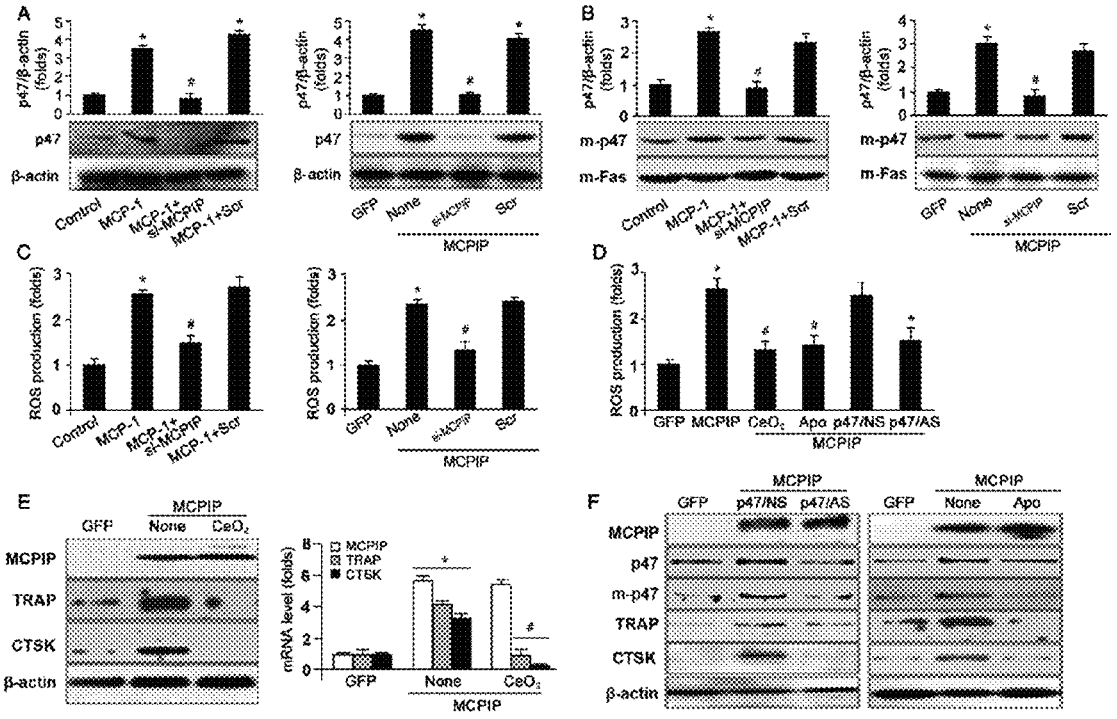
MCPIP
TRAP
CTSK
RNA level (folds)
Control
GFP
1
2
4
6 (days)
MCPIP
αV
β3
MMP9
B
Protein level (folds)
GFP
None
si-MCPIP
Scr
C
TRAP-positive cells (%)

Figure 3



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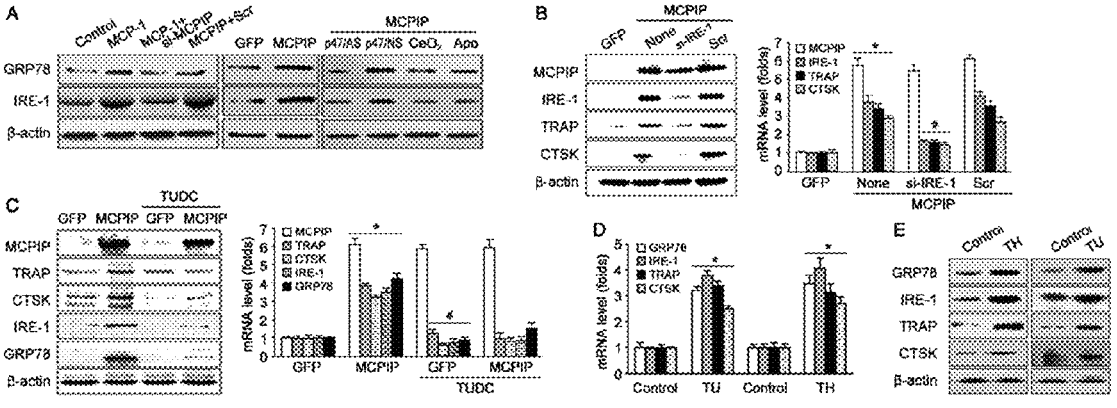


Figure 5

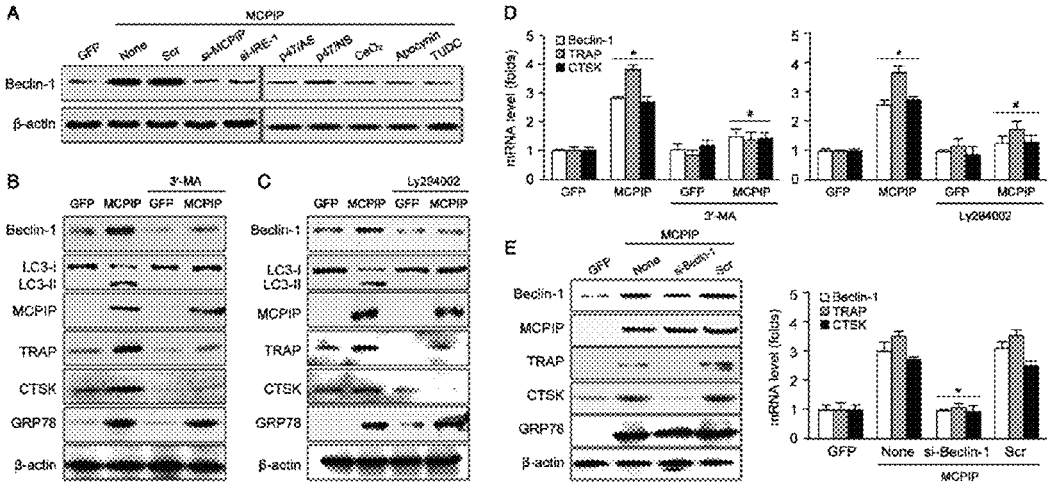
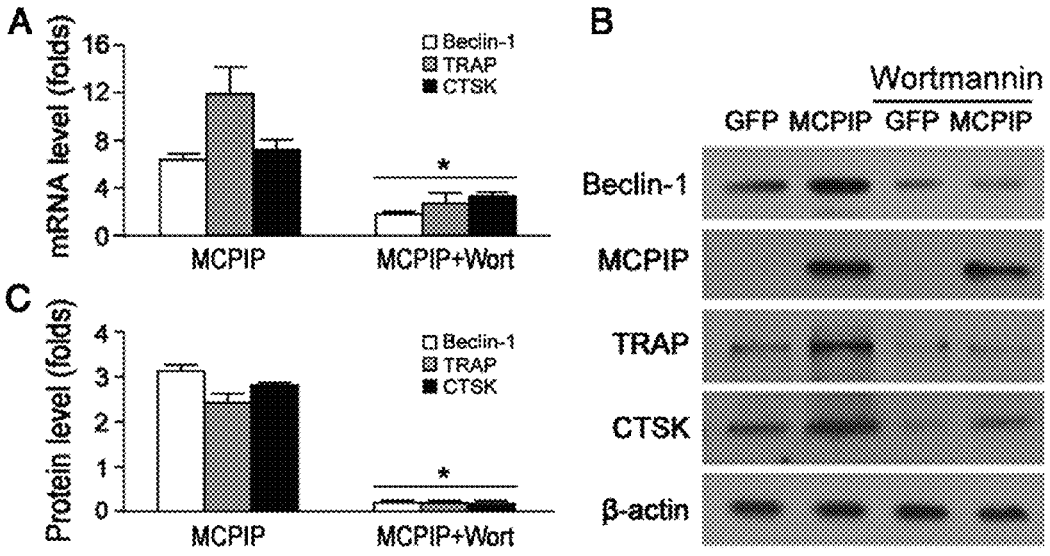


Figure 6



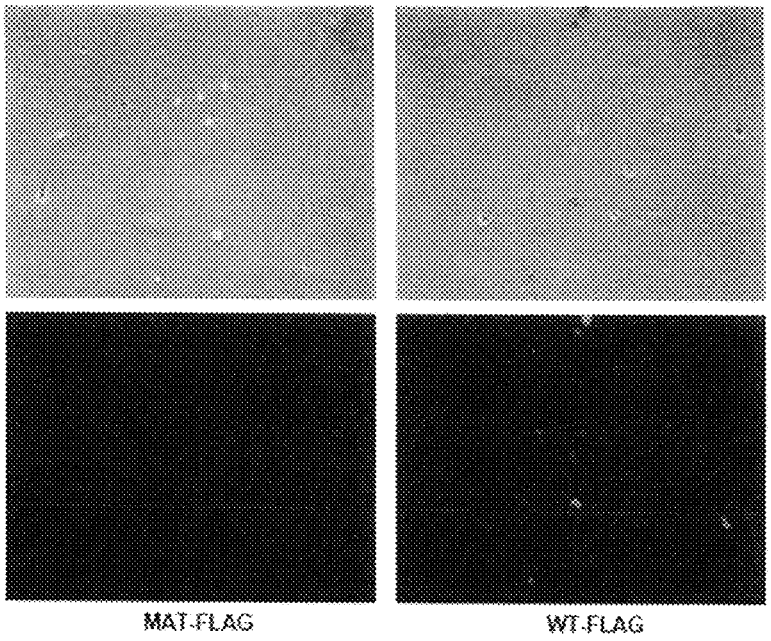
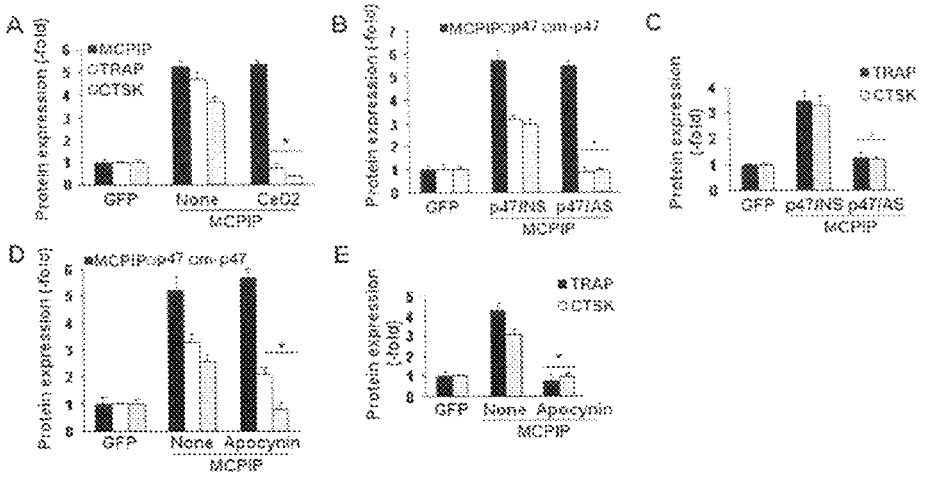


FIG. 7


A
Protein expression (-fold)
MCPIP
TRAP
CTSK
GFP
None
Ce02
MCPIP
B
Protein expression (-fold)
MCPIP
p47 or n-p47
GFP
p47NS
p47AS
MCPIP
C
Protein expression (-fold)
TRAP
CTSK
GFP
p47NS
p47AS
MCPIP
D
Protein expression (-fold)
MCPIP
p47 or n-p47
GFP
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Apocynin
MCPIP
E
Protein expression (-fold)
TRAP
CTSK
GFP
None
Apocynin
MCPIP

FIG. 8

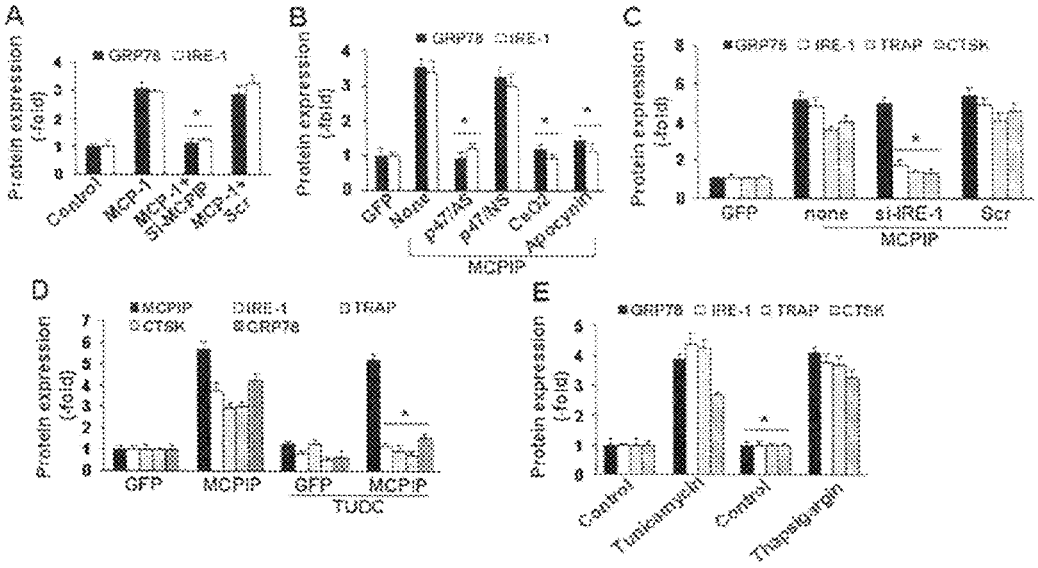
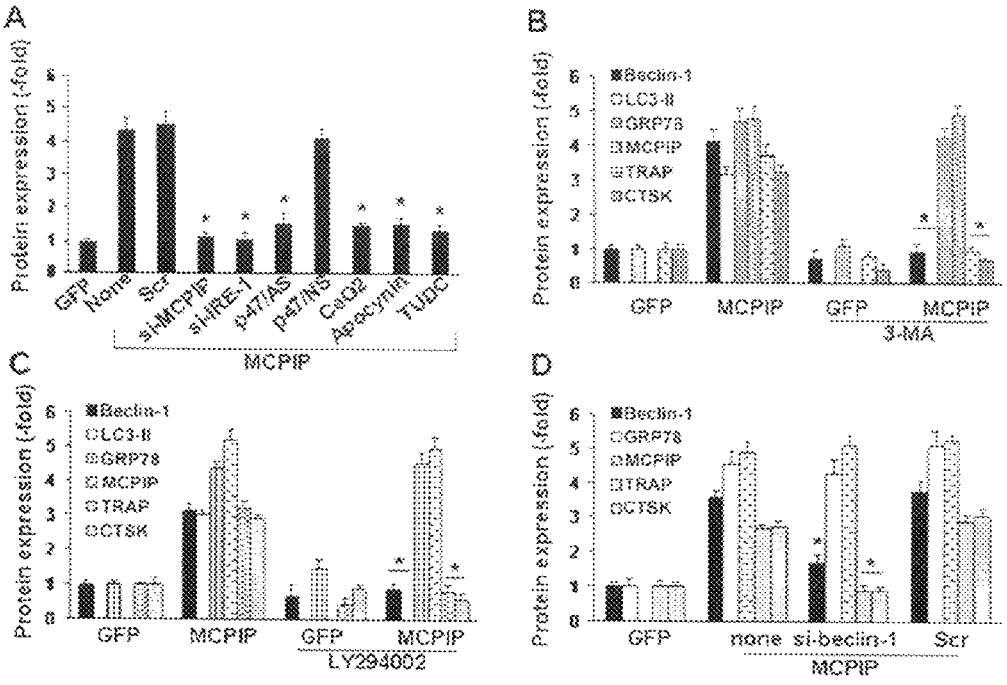

A
B
C
D
E
Protein expression (fold)
GRP78
IRE-1
TRAP
CTSK
MCPIP
Control
MCP-1
MCP-1+ SiMCPIP
MCP-1+ Scr
GFP
Noco
p47/A5
p47/N5
CeCa
Apocynin
none
siIRE-1
Scr
TUDC
Tunicamycin
Thapsigargin

FIG. 9


A
Protein expression (-fold)
GFP
None
Scr
si-MCPIP
si-IRE-1
p47/AS
p47/NS
CaQ2
Agacynin
TUDC
MCPIP
B
Protein expression (-fold)
Beclin-1
LC3-II
GRP78
MCPIP
TRAP
CTSK
GFP
MCPIP
GFP
MCPIP
3-MA
C
Protein expression (-fold)
Beclin-1
LC3-II
GRP78
MCPIP
TRAP
CTSK
GFP
MCPIP
GFP
MCPIP
LY294002
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CTSK
GFP
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si-beclin-1
Scr
MCPIP

FIG. 10

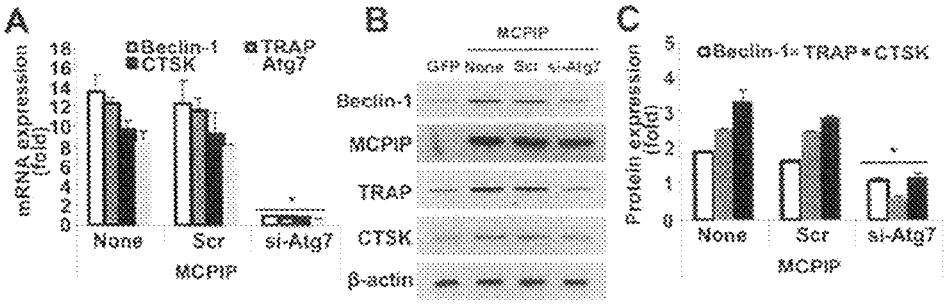
A
mRNA expression (fold)
Beclin-1
CTSK
TRAP
Atg7
None
Scr
si-Atg7
MCPIP
B
MCPIP
GFP None Scr si-Atg7
Beclin-1
MCPIP
TRAP
CTSK
β-actin
C
Protein expression (fold)
Beclin-1 TRAP CTSK
None
Scr
si-Atg7
MCPIP

FIG. 11

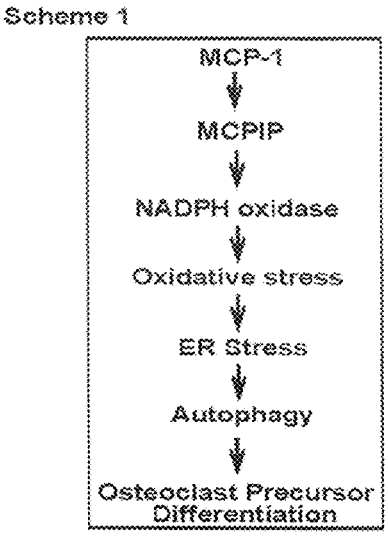
Scheme 1
MCP-1
MCPIP
NADPH oxidase
Oxidative stress
ER Stress
Autophagy
Osteoclast Precursor Differentiation

FIG. 12

MCPIP PROTECTION AGAINST OSTEOCLAST PRODUCTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 13/362,261, filed Jan. 31, 2012 now abandoned, and is a continuation of U.S. Ser. No. 12/539,907 filed Aug. 12, 2009, now abandoned, which is a Continuation of Ser. No. 11/643,057; filed Dec. 20, 2006, now abandoned, which claims benefit of U.S. Ser. No. 60/751,927 filed Dec. 20, 2005; all of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Apr. 12, 2012, is named 10669150.txt and is 20,777 bytes in size.

BACKGROUND

There is strong evidence that osteoclast (OC) is the principal cell type responsible for bone resorption in inflammatory joint diseases (Harris, 1990; Sakiyama et al., 2001; Sato and Takayanagi, 2006; Mundy, 2007). Rheumatoid arthritis (RA) is characterized by the presence of inflammatory synovitis accompanied by the destruction of the joint cartilage and bone (Harris, 1990; Mundy, 2007; Sugimura and Li, 2010). OCs are bone-resorbing cells that differentiate from hematopoietic precursors of the monocyte/macrophage lineage (Sakiyama et al., 2001; Boyce et al., 2007). OCs are multinuclear giant cells that stain positive for tartrate-resistant acid phosphatase (TRAP) and serine protease cathepsin K (CTSK) (Kiviranta et al., 2001; Boyce et al., 2007).

Monocyte chemotactic protein-1 (MCP-1), a CC chemokine commonly found at the site of tooth eruption, RA bone degradation, and bacterially induced bone loss (Wise et al., 2002), is known to induce differentiation of monocytes into TRAP and CTSK-positive precursors of OCs. MCP-1 is expressed by mature OCs and its expression is regulated by nuclear factor-κB (NF-κB) (Kim et al., 2005). Several reports showed that MCP-1 is induced by NF-κB ligand RANKL and promotes OC fusion into multinuclear TRAP-positive cells without bone-resorption activity (Kim et al., 2006a,b), which might be called OC precursors. Recently, it has been reported that MCP-1 plays an important role in regulating OC differentiation in an autocrine/paracrine manner under stimulation by RANKL (Miyamoto et al., 2009). How MCP-1 mediates OC differentiation remains unclear.

The cellular effect of MCP-1 is mediated by the CCR2, a G-protein-coupled receptor that is induced by the receptor activator of RANKL (Gerszten et al., 2001; Kim et al., 2005). The signaling process initiated by MCP-1 binding to CCR2 leads to changes in gene expression.

Recently, it was found that this MCP-1 binding leads to the induction of a novel zinc-finger protein called MCPIP in human peripheral blood monocytes (Zhou et al., 2006). The biological functions of MCPIP, however, remain poorly understood.

SUMMARY

The importance of the way in which MCP-1 mediates OC differentiation has been realized, which has been heretofore unclear. The role of MCPIP in biological processes initiated by MCP-1 has also now been discovered. It was previously understood that MCPIP mediates several biological processes initiated by MCP-1. including cardiomyocyte death (Younce and Kolattukudy, 2010; Younce et al., 2010), adipogenesis (Younce et al., 2009), angiogenesis (Niu et al., 2008), and glial differentiation of neuroprogenitor cells (Vrotsos et al., 2009). It has been discovered, as disclosed herein, that MCP-1 induces differentiation of monocytic cells into OC precursors via MCPIP. It is therefore presented that MCPIP mediates differentiation of OC precursors via induction of oxidative stress that causes endoplasmic reticulum (ER) stress that leads to autophagy involved in osteoclastogenesis. These findings implicate that MCPIP is a novel factor involved in OC precursor differentiation, and thus it serves as a new target for diagnosis and treatment of osteoporosis-related disease.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**. MCP-1 induction of OC precursor differentiation, mediated by MCPIP. (A and B) BMCs were transfected with FLAG-tagged MCPIP (WT-MCPIP) or FLAG-tagged empty vector (MAT-FLAG). TRAP-expressing cells were stained and viewed under the Nikon microscope (A). BMNCs treated with MCP-1 alone or with MCPIP siRNA or Scr siRNA. TRAP-expressing cells were stained and TRAP-positive cell proportion was measured (B). At least three fields (~500 cells), were chosen. *P<0.05 and **P<0.01 versus control; #P<0.05 versus Scr. (C) Immunoblotting showing the expression of TRAP, CTSK, and MCPIP induced by MCP-1. Data were mean±SD (n=3). *P<0.05 versus control; #P<0.05 versus Scr. (D) Real-time PCR showing the transcription of TRAP, CTSK, and MCPIP induced by MCP-1. *P<0.05 versus control; #P<0.05 versus Scr. (E) Real-time PCR showing the transcription of aV integrin, β3 integrin, and MMP9.

FIG. **2**. MCPIP overexpression induces OC-related marker TRAP and CTSK expression. (A) Real-time PCR showing the transcription of TRAP, CTSK, and MCPIP (upper panel) and αV integrin, β3 integrin, and MMP9 (lower panel) induced by MCPIP transfection. *P<0.05 and **P<0.01 versus control or GFP; #P<0.05 versus day 2 or 6. (B) Immunoblotting showing the expression of TRAP, CTSK, and MCPIP induced by MCPIP transfection. Data were mean±SD (n=3). *P<0.01 versus GFP; #P<0.05 versus MCPIP or Scr. (C) Percentage of TRAP-positive cells. *P<0.01 versus GFP; #P<0.01 versus none or Scr.

FIG. **3**. ROS production involvement in MCPIP/MCP-1-induced OC precursor differentiation. (A) Western blot showed that MCP-1 treatment or MCPIP transfection induces $p47^{PHOX}$ expression. *P<0.05 versus control or GFP; #P<0.05 versus Scr. (B) Immunoblotting showed that MCP-1 or MCPIP overexpression induces an increase in cytoplasmic membrane-associated $p47^{PHOX}$. *P<0.05 versus control or GFP; #P<0.05 versus Scr. (C) ROS production induced by MCP-1 or MCPIP transfection, or siRNA was detected by using DHR123. *P<0.05 versus control or GFP; #P<0.05 versus Scr. (D) MCPIP-induced ROS production was inhibited by ROS, NAD(P)H oxidase inhibitors, and $p47^{PHOX}$ knockdown. *P<0.05 versus GFP, #P<0.05 versus MCPIP only. *P<0.05 compared with $p47^{PHOX}$ non-specific (NS) oligonucleotides transfection cells. (E) The effect of CeO_2 on MCPIP, TRAP, and CTSK expression induced by MCPIP transfection. Data were mean±SD (n=3). *P<0.05 versus GFP; #P<0.05 versus MCPIP only. (F) The effect of $p47^{PHOX}$ AS and apocynin on the expression of related

proteins. m-p47, membrane located $p47^{PHOX}$; p47, total $p47^{PHOX}$; p47/NS, non-specific RNA; p47/AS, antisense RNA. Apo, apocynin.

FIG. **4**. MCPIP induction of ER stress via ROS production involved in OC precursor differentiation. (A) Immunoblotting shown that MCP-1 or MCPIP transfection induces the expression of GRP78 and IRE-1 (ER stress markers) by inducing ROS production. (B) Blockage of ER stress by IRE-1 siRNA abolished MCPIP-induced mRNA and protein expression of TRAP and CTSK. *P<0.05 versus GFP, #P<0.05 versus Scr. (C) Blockage of ER stress by TUDC abolished MCPIP-induced mRNA and protein expression of TRAP and CTSK. *P<0.05 versus GFP, #P<0.05 versus MCPIP alone. (D) Real-time PCR showing the expression of TRAP, CTSK, GRP78, and IRE-1 by ER stress inducer. *P<0.05 versus control. (E) Immunoblotting showing the expression of TRAP, CTSK, GRP78, and IRE-1 by ER stress inducer. TU, tunicamycin; TH, thapsigargin.

FIG. **5**. MCPIP induction of autophagy via ROS production and ER stress involved in OC precursor differentiation. (A) Immunoblotting shown that MCPIP induces autophagy characterized with the marker Beclin-1 expression and ROS/ER stress inhibitor inhibited MCPIP-induced expression of Beclin-1. (B and C) Immunoblotting shown that autophagy blocker 3'-MA and LY294002 blocked MCPIP-induced OC-related gene TRAP and CTSK expression but not GRP78. (D) Real-time PCR shown that autophagy blocker 3'-MA and LY294002 blocked MCPIP-induced expression of TRAP and CTSK but not GRP78. *P<0.05 versus GFP, #P<0.05 versus MCPIP alone. (E) Blocking autophagy by Beclin-1 siRNA inhibited MCPIP-induced mRNA and protein expression of TRAP and CTSK but not GRP78. *P<0.05 versus MCPIP alone.

FIG. **6**. MCPIP-induced OC precursor differentiation inhibited by PI3K inhibitor, wortmannin. (A) qRT-PCR showing induction of Beclin-1 and TRAP and CTSK at the transcript level by MCPIP transfection, and the inhibition effect of wortmannin on MCPIP. *P<0.05 versus MCPIP alone. (B) Immunoblotting show that MCPIP induced Beclin-1, and TRAP and CTSK, and that their induction was suppressed by wortmannin. (C) The intensities of immunoblots were measured and normalized to β-actin of the corresponding group. *P<0.05 compared with MCPIP alone.

FIG. **7**. MCPIP induced production of cellular ROS in bone marrow mononuclear cells (BBMN). Cells were transfected with empty vector (MAT-FLAG) orMCPIP-expression vector (WT-MCPIP) and after 24 hr stained with DHR123 that is a cell permeable nonfluorescent reagent that generates a fluorescent product (red) when oxidized by cellular ROS.

FIG. **8**. ROS production involvement in MCPIP/MCP-1 induced OC precursor differentiation. Bone marrow monocytes were pretreated with CeO2 or apocynin for 6 h or p47PHOXantisense oligonucleotides for 24 h and then cells were transfected with MCPIP or GFP for 4 days. Cell lysate was collected and analysed using immunoblot with appropriate antibody and results were quantified against β-actin. A, Effect of CeO2 on MCPIP-induced TRAP and CTSK expression [*P<0.05 compared to CeO2-untreated cells ("None")]. B, Effect of p47 PHOX antisense oligonucleotides on expression of p47 PHOX and its translocation from cytoplasm to membrane [*P<0.05 compared to non-sense oligonucleotides treated cells ("p47/NS")]. C, Effect of p47 PHOX antisense oligonucleotides on MCPIP-induced TRAP and CTSK expression [*P<0.05 compared to non-sense oligonucleotides treated cells ("p47/NS")]. D, Effect of apocynin on expression of p47PHOX and its translocation from cytoplasm to membrane [*P<0.05 compared to apocynin-untreated cells ("None")]. E, Effect of apocynin on MCPIPinduced TRAP and CTSK expression [*P<0.05 compared to apoicynin-untreated cells ("None")].

FIG. **9**. MCPIP induces ER stress via ROS production which involvement in OC precursor differentiation. After treatment, cell lysate was collected and analysed using immunoblot with appropriate antibody and results were quantified against β-actin. A, MCP-1 induces ER stress marker GRP78 and IRE-1 expression via MCPIP (*P<0.05). B, Effect of p47PHOX antisense oligonucleotides, Ce02 and apocynin on MCPIP-induced expression of GRP78 and IRE-1 (*P<0.05). C, inhibition of ER stress by using knockdown of IRE-1 attenuates MCPIP-induced expression of TRAP and CTSK (*P<0.05). D, pretreatment of TUDC inhibits MCPIP-induced expression of TRAP and CTSK and ER stress marker IRE-1 and GRP78 (* P<0.05). E, ER stress inducer tunicamycin and thapsigargin induce expression of TRAP and CTSK (*P<0.05).

FIG. **10**. MCPIP induces autophagy via ROS production and ER stress which involvement in OC precursor differentiation. After treatment, cell lysate was collected and analysed using immunoblot with appropriate antibody and results were quantified against β-actin. A, MCPIP induces autophagy characterized with the marker beclin-1 expression and ROS inhibitor or ER stress inhibitor inhibited MCPIP-induced expression of beclin-1. (*P<0.05). B, autophagy blocker 3'-MA blocked MCPIP-induced osteoclast-related gene TRAP and CTSK expression but not GRP78 (*P<0.05). C, autophagy blocker LY-294002 blocked MCPIP induced osteoclast-related gene TRAP and CTSK expression but not GRP78. (*P<0.05). D, blockage of autophagy by beclin-1 specific siRNA inhibited MCPIP-induced osteoclast-related gene TRAP and CTSK expression but not GRP78 both in protein and mRNA level. *P<0.05.

FIG. **11** MCPIP-induced autophagy marker Atg7 is necessary for induction of OC precursor differentiation. (A) qRT-PCR shows that transfection of MCPIP expression vector resulted in significantly increased mRNA levels of Atg7, Beclin-1, TRAP, and CTSK. Atg7-specific siRNA abolished MCPIP-induced expression of these genes. *P<0.05 compared with MCPIP alone. (B) Immunoblot shows that transfection with MCPIP expression vector caused induction of beclin-1, TRAP, and CTSK at the protein levels. Upregulation of these marker genes were inhibited by Atg7-specific siRNA but were not affected by scrambled siRNA. (C) The intensity of each protein was measured and normalized to β-actin of the corresponding group. *P<0.05 compared with MCPIP alone.

FIG. **12** Supplementary Scheme This figure shows a possible mechanism of how MCP-1 might effect Osteoclast precursor Differentiation

DETAILED DESCRIPTION

The inventors previously identified the novel transcription factor designated as MCPIP (MCP-1-induced protein). MCPIP was initially isolated from human monocytes after stimulation with MCP-1. The nucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences of isolated human MCPIP were deposited with GenBank under accession number AY920403 and the nucleotide (SEQ ID NO: 3) and amino acid (SEQ ID NO: 4) sequences of isolated mouse MCPIP were deposited with GenBank under accession number AY920404.

The inventors have continued to study the biological relevance of these genes/proteins, and to develop new therapies based on this research. This invention is based on the discovery that MCP-1 induces MCPIP in human peripheral blood monocytes which induces differentiation of monocytes into osteoclast cells (OC) or OC precursors. NF_KB is a master controller of inflammation in the body. Agonists of MCPIP would enhance its anti-inflammatory potency; it is herein discovered that inhibition of the activation of NF_KB by MCPIP provides a significant potential as an anti-inflammatory agent. Therefore, inhibition of the activation of NF_KB by inhibiting MCPIP has a significant use as an anti-inflammatory agent. Inhibition of MCPIP inhibits inflammation, which plays a critical role in diseases including angiogenesis, i.e., the physiological process involving the growth of new blood vessels from pre-existing vessels, adipogenesis, the process of cell differentiation by which preadipocytes become adipocytes, and osteoclastogenesis, i.e., the development of osteoclasts. Furthermore, MCPIP plays a critical role in the differentiation of osteoclasts involved in inflammatory bone loss that occurs in many inflammatory diseases such as, for example, rheumatoid arthritis. Additionally, elevated MCP-1 levels have been identified as a direct cause of insulin resistance. MCPIP has been discovered to mediate insulin resistance, and thus, MCPIP inhibitors enhance insulin sensitivity. Additional research has led to the identification of MCPIP as a novel factor involved in OC precursor differentiation, as provided herein, identifies MCPIP as a new target for diagnosis and treatment of osteoporosis-related disease.

Discovered herein is the effect of inhibition of MCPIP in reducing osteoclast precursor differentiation. MCPIP plays a critical role in differentiation of osteoclasts involved in inflammatory bone loss that occurs in many inflammatory diseases such as rheumatoid arthritis. It is disclosed herein that differentiation of monocytes to OC precursors is mediated by MCPIP. Therefore, the blocking or inhibition of MCPIP provides a promising therapeutic strategy for preventing differentiation of monocytes to OC precursors, and ultimately the prevention of bone resorption. Bone resorption is a process by which osteoclasts break down bone and release minerals, resulting in a transfer of calcium from bone fluid to the blood. This discovery provides a particular benefit in inflammatory joint diseases.

Ultimately, inflammatory bone erosion is involved in many pathological conditions (Lu et al., 2007; Ha et al., 2010). The novel inventive features described and contemplated herein provide a new insight into the mechanism by which MCP-1 induces differentiation of monocytic cells into TRAP- and CTSK-expressing cells that can proceed to differentiate into functional OCs in the presence of RANKL, and demonstrate that MCPIP is a novel target for therapy of inflammatory bone erosion, in one exemplary embodiment.

According to one embodiment of the invention, a method of treating a condition in a patient in need includes administering to the patient a therapeutically effective amount of a composition that inhibits the expression or action of MCPIP. The patient in need may be exhibiting pre-arthritic symptoms including but not limited to pain or tenderness in a joint which is aggravated by movement or activity, inflammation indicated by joint swelling, stiffness, redness, and/or warmth, joint deformity, loss of range of motion or flexibility in a joint, or unexplained weight loss. Additional pre-arthritic symptoms include extreme fatigue, lack of energy, weakness or a feeling of malaise, a non-specific fever, or crepitus, i.e., creaky, popping or snapping joints.

In another embodiment, the patient in need may be exhibiting pre-osteoporotic symptoms, including but not limited to back pain caused by a fractured or collapsed vertebra, loss of height over time, a stooped posture, or a bone fracture that occurs much more easily than expected. The patient in need may also be exhibiting symptoms of an existing arthritic or osteoporotic disease, or both. Furthermore, the patient in need may be exhibiting symptoms of any arthritic or osteoporotic related-diseases, any inflammatory joint diseases, or any pre-disease symptoms of these related diseases. In a further embodiment, the condition may include an osteoporosis-related condition, and in a particular embodiment, rheumatoid arthritis.

As used herein, therapeutically effective amount refers to an amount sufficient to elicit the desired biological response. In the present invention the desired biological response can be an overall improvement in the condition being treated. The overall improvement can be associated with improvement in individual symptoms.

Subject or patient, as used herein, refers to animals such as mammals, including, but not limited to, primates (e.g., humans), cows, sheep, goats, horses, pigs, dogs, cats, rabbits, guinea pigs, rats, mice or other bovine, ovine, equine, canine, feline, rodent or murine species. In one embodiment, the subject is a human.

Modes of Administration

The compounds for use in the method of the invention can be formulated for oral, transdermal, sublingual, buccal, parenteral, rectal, intranasal, intrabronchial, intrapulmonary, or ocular administration. Oral administration is preferred. For oral administration, the compounds can be of the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., polyvinylpyrrolidone, hydroxypropylcellulose or hydroxypropylmethylcellulose); fillers (e.g., cornstarch, lactose, microcrystalline cellulose or calcium phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrates (e.g., sodium starch glycollate); or wetting agents (e.g., sodium lauryl sulphate). If desired, the tablets can be coated using suitable methods and coating materials such as OPADRY film coating systems available from Colorcon, West Point, Pa. (e.g., OPADRY, OY Type, OY-C Type, Organic Enteric OY-P Type, Aqueous Enteric OY-A Type, OY-PM Type and OPADRY® White, 32K18400).

In a particular embodiment, the oral form is a tablet containing MCPIP and a pharmaceutically acceptable excipient, such as, but not limited to mannitol, corn starch, microcrystalline cellulose, colloidal silicon dioxide, polyvinyl pyrrolidone, talc, magnesium stearate, and the like which are optionally coated with an OPADRY film coating.

Liquid preparation for oral administration can be in the form of solutions, syrups or suspensions. The liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agent (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); and preservatives (e.g., methyl or propyl p-hydroxy benzoates or sorbic acid).

For buccal administration, the compounds for use in the method of the invention can be in the form of tablets or lozenges formulated in a conventional manner.

For parenteral administration, the compounds for use in the method of the invention can be formulated for injection or infusion, for example, intravenous, intramuscular or subcutaneous injection or infusion, or for administration in a bolus dose and/or continuous infusion. Suspensions, solu-

tions or emulsions in an oily or aqueous vehicle, optionally containing other formulatory agents such as suspending, stabilizing and/or dispersing agents can be used.

For rectal administration, the compounds for use in the method of the invention can be in the form of suppositories or enemas. For sublingual administration, tablets can be formulated in conventional manner.

For intranasal, intrabronchial or intrapulmonary administration, conventional formulations can be employed.

Further, the compounds (e.g. protein or delivery vehicle) for use in the method of the invention can be formulated in a sustained release preparation. For example, the compounds can be formulated with a suitable polymer or hydrophobic material which provides sustained and/or controlled release properties to the active agent compound. As such, the compounds for use the method of the invention can be administered in the form of microparticles for example, by injection or in the form of wafers or discs by implantation.

In accordance with the method of the invention, an expression vector is a viral or a non-viral expression vector. Viral expression vectors which may be used advantageously in the method of the invention include, but are not limited to, an adeno associated virus (AAV) vector, a lentivirus vector, an adenovirus vector, and a herpes simplex virus (HSV) vector.

In additional embodiments, the composition comprises siRNA or miRNA specific for MCPIP, an antisense nucleotide specific for MCPIP, and/or shRNA. In an alternative embodiment, the composition comprises an antibody specific to MCPIP.

In another embodiment, a method of inhibiting osteoclast production (or a method of reducing osteoclast precursor cells) in a patient in need is provided. The method includes administering a therapeutically effective amount of a composition that inhibits the expression or action of MCPIP in the patient. The patient in need, in an embodiment, may be exhibiting symptoms of rheumatoid arthritis, osteoarthritis, and/or osteoporosis.

In another embodiment, administering a therapeutically effective amount of a composition includes a composition comprising: a composition that inhibits the expression or action of MCPIP, and a pharmaceutically acceptable excipient.

In further embodiments, the composition includes an MCPIP siRNA, an shRNA, an antibody specific to MCPIP, and/or an antisense nucleotide specific for MCPIP.

Many of the embodiments of the subject invention make reference to particular methods of inhibiting expression. The subject invention is not to be limited to any of the particular methods described. One such method includes siRNA (small interfering/short interfering/silencing RNA). SiRNA most often is involved in the RNA interference pathway where it interferes with the expression of a specific gene. In addition to its role in the RNA interference pathway, siRNA also act in RNA interference-related pathways, e.g., as an antiviral mechanism or in shaping the chromatin structure of a genome.

Another method by which to inhibit expression and to inhibit the expression of MCPIP in particular is shRNA. ShRNA (short hairpin or small hairpin RNA) refers to a sequence of RNA that makes a tight hairpin turn and is used to silence gene expression via RNA interference. It uses a vector introduced into cells and a U6 or H1 promoter to ensure that the shRNA is always expressed. The shRNA hairpin structure is cleaved by cellular machinery into siRNA which is then bound to the RNA-induced silencing complex. This complex binds to and cleaves mRNAs which match the siRNA that is bound to it.

MCPIP can also be blocked by subjecting procured cells to an antibody specific to MCP-1. An antisense nucleotide may also be used to block or inhibit expression, in particular, the expression of MCP-1. Expression may also be inhibited with the use of a morpholino oligomer or phosphorodiamidate morpholino oligomer (PMO). PMOs are an antisense technology used to block access of other molecules to specific sequences within nucleic acid. PMOs are often used as a research tool for reverse genetics, and function by knocking down gene function. This is achieved by preventing cells from making a targeted protein or by modifying splicing of pre-mRNA.

EXAMPLES

Example 1

MCP-1 Induces OC-Related Gene Expression Via MCPIP in Human Bone Marrow Monocytes

It has been demonstrated that MCP-1 induces TRAP-positive OC precursor formation from human peripheral blood mononuclear cells (Kim et al., 2006a). Here, we found that 50 ng/ml MCP-1 induced TRAP-positive OC precursor cell formation from human bone marrow mononuclear cells (BMCs) (FIG. **1**B). Immunoblotting (FIG. **1**C) and real-time polymerase chain reaction (PCR) (FIG. **1**D) showed that MCP-1 treatment induced expression of OC markers TRAP and CTSK. However, MCP-1 did not affect the expression of the OC functional markers αV integrin, β3 integrin, and MMP9 (FIG. **1**E). We also found that 50 ng/ml MCP-1 induced up-regulation of MCPIP protein and mRNA levels, which can be suppressed by treatment with MCPIP small interfering RNA (siRNA) (FIGS. **1**C and D). Compared with FLAG-tagged empty vector (MAT-FLAG), expression of FLAG-tagged MCPIP (WT-FLAG) induced TRAP-positive OC precursor cell formation (FIG. **1**A). MCPIP siRNA also significantly inhibited the formation of OC precursor cells that expressed TRAP and CTSK but showed no effects on expression of αV integrin, β3 integrin, and MMP9 (FIG. **1**C-E). These results suggest that induction of the TRAP-positive OC precursor cells by MCP-1 treatment of BMCs was mediated via MCPIP.

Example 2

Forced Expression of MCPIP Induces Differentiation of Monocytes into OC Precursors

If MCP-1-induced differentiation of OC precursors is mediated by MCPIP, forced expression of MCPIP in monocytes might be expected to induce the formation of TRAP-positive OC precursor cells without MCP-1. BMCs were transfected to test this concept, as can be seen in FIG. **2**, with MCPIP-GFP expression vector. Increased expression of MCPIP was found 24 h after transfection at mRNA levels as measured by real-time PCR and its expression reached the peak at 4 days after transfection (see FIG. **2**A, upper panel). The expression of TRAP and CTSK was induced at 2 days after MCPIP transfection and reached the peak at 4 days. However, the expression of αV integrin, β3 integrin, and MMP9 showed no significant changes after MCPIP transfection (see FIG. **2**A, bottom panel). Immunoblot analysis showed that MCPIP overexpression induced expression of TRAP and CTSK (see FIG. **2**B), and TRAP staining showed

that MCPIP expression significantly elevated formation of TRAP-positive cells (FIG. 2C). When MCPIP siRNA was transfected into BMCs for 24 h prior to MCPIP transfection, MCPIP expression was knocked down and the expression of OC-related genes TRAP and CTSK also were down-regulated (FIG. 2B). Moreover, the percentage of TRAP-positive cells was lowered by treatment with MCPIP siRNA (FIG. 2C). These results suggest that MCPIP transfection causes induction of OC-related genes and formation of TRAP-positive OC precursor cells.

Example 3

MCPIP-Induced Reactive Oxygen Species Production is Involved in OC Precursor Differentiation

Reactive oxygen species (ROS) derived from NADPH oxidase have been suggested to regulate OC differentiation and prolong the survival of OC precursors (Yamasaki et al., 2009). p47PHOX, a regulatory subunit of NADPH oxidase, has been implicated in ROS generation (Decoursey and Ligeti, 2005). Herein, it was tested whether forced expression of MCPIP could induce expression and activation of NADPH oxidase by translocation of p47PHOX into the membrane and produce ROS. Dihydrorhodamine 123 (DHR123) staining revealed ROS production by MCPIP transfected cells but not MAT-FLAG controls (FIG. 7). Immunoblot analysis shows that MCP-1 treatment and MCPIP expression increased the expression of p47PHOX (FIG. 3A) and its translocation from the cytoplasm into the membrane (FIG. 3B). ROS production caused by MCP-1 treatment and MCPIP expression was assessed by DHR123 staining and results showed that MCP-1 treatment and MCPIP transfection remarkably increased ROS generation. Moreover, the effects of MCP-1 treatment or forced MCPIP expression on the expression and translocation of p47PHOX and ROS generation were inhibited by MCPIP siRNA (FIG. 3A-C).

To understand the involvement of ROS production in MCPIP-mediated formation of TRAP-positive OC precursor cells, BMCs were treated with CeO_2 nanoparticles, an inhibitor of ROS (Tsai et al., 2007) prior to MCPIP transfection. It was found that MCPIP-induced ROS production was significantly inhibited by CeO_2 (FIG. 3D). Immunoblot and real-time PCR analysis showed that CeO_2 inhibited MCPIP-induced TRAP and CTSK expression both at protein and mRNA levels (FIG. 3E and FIG. 8A). The effect of apocynin, an inhibitor of NADPH oxidase on MCPIP-induced induction of OC-related genes TRAP and CTSK was further tested herein. It was found that apocynin suppressed MCPIP-induced ROS production (FIG. 3D) and expression of TRAP and CTSK (FIG. 3F and FIG. 8E). Moreover, apocynin inhibited MCPIP-induced expression and membrane translocation of $p47^{PHOX}$ (FIG. 3F and FIG. 8D). Furthermore, knock-down of $p47^{PHOX}$ by its specific antisense oligonucleotides (p47/AS) also decreased ROS production, expression and translocation of $p47^{PHOX}$, and expression of OC-related genes TRAP and CTSK (FIG. 3F and FIGS. 8B and C). These results suggest that MCPIP causes ROS production by up-regulating $p47^{PHOX}$ expression and its membrane translocation, and that ROS generation is involved in MCP-1-induced OC precursor differentiation.

Example 4

MCPIP-Induced ROS Production Causes an ER Stress Response that is Involved in OC Precursor Differentiation

It has been reported that oxidative stress can induce ER stress (Xue et al., 2005; Malhotra et al., 2008). Therefore, it was tested herein whether MCP-1 treatment induces ER stress in BMCs. Immunoblot analysis showed that MCP-1 treatment induced expression of the ER stress markers 78 kDa glucose regulated protein (GRP78) and inositol-requiring enzyme-1 (IRE-1) (FIG. 4A, left panel, and FIG. 9A). This induction was inhibited by MCPIP siRNA (FIG. 4A, left panel, and FIG. 9A), indicating that MCP-1 induced ER stress via MCPIP. It was also found that forced expression of MCPIP induced ER stress with up-regulation of GRP78 and IRE-1 as indicated by immunoblot (FIG. 4A and FIG. 9B). To test whether MCPIP induces ER stress through oxidative stress, ROS production was suppressed by CeO_2, apocynin, and p47/AS, and then immunoblotting was performed. Results showed that MCPIP-induced expression of GRP78 and IRE-1 was attenuated by CeO_2, apocynin, and p47/AS (FIG. 4A and FIG. 9B). These results suggest that MCP-1 mediated ER stress via MCPIP-induced ROS production.

To understand whether ER stress is involved in MCPIP-induced expression of OC-related genes TRAP and CTSK, MCPIP-induced ER stress was inhibited by IRE-1 siRNA (FIG. 4B and FIG. 9C) and the ER stress-specific inhibitor tauroursodeoxycholate (TUDC) (FIG. 4C and FIG. 9D). Immunoblot and real-time PCR analysis showed that MCPIP-induced expression of TRAP and CTSK was significantly inhibited by IRE-1 siRNA and by TUDC at both protein and mRNA levels but did not affect the expression of MCPIP (FIGS. 4B and C).

If ER stress is critically important for OC differentiation, the inventors identified that ER stress inducers might induce differentiation of OC precursors without other inducers. In fact, two ER stress inducers, tunicamycin (TU) and thapsigargin (TH), induced expression of GRP78, IRE-1, TRAP, and CTSK at both protein and mRNA levels (FIGS. 4D and E and FIG. 9E). This result discovered by the inventors suggests that induction of ER stress alone could induce OC precursor differentiation. These results strongly support the conclusion that MCPIP-induced ER stress is involved in MCP-1-mediated OC precursor differentiation.

Example 5

MCPIP-Induced Oxidative and ER Stress Leads to Autophagy Involved in OC Precursor Differentiation

Increased expression of Beclin-1 is a commonly used marker of autophagy (Wang, 2008). Autophagy has been implicated in differentiation in some cellular contexts (Baerga et al., 2009; Singh et al., 2009). However, it was heretofore unclear whether autophagy has involvement in OC differentiation. Here it was found that forced expression of MCPIP increased expression of Beclin-1; this effect was suppressed by MCPIP siRNA, but not by non-specific scramble (Scr) siRNA (FIG. 5A, left panel and FIG. 10A). This result revealed that MCPIP induced autophagy in BMCs during differentiation into OC precursor cells.

ER stress is known to induce autophagy. To test whether MCPIP-induced ROS production and ER stress are involved

directly in MCPIP-mediated autophagy, MCPIP-expressing cells were treated with CeO_2 nanoparticles that can trap free radicals, NADPH inhibitor apocynin, p47/AS, ER stress inhibitor TUDC, or IRE-1 siRNA. Immunoblot analysis showed that MCPIP-induced expression of Beclin-1 was inhibited significantly by inhibition of oxidative stress and ER stress and knockdown of genes involved in these stresses (FIG. **5**A, right panel, and FIG. **10**A). These results suggested that MCPIP-mediated autophagy was caused by oxidative stress and ER stress during differentiation of BMCs into OC precursor cells.

Recently, it has been shown that the PI3K inhibitors LY294002 and 3'-methyladenine (MA) stop the macroautophagic pathway at the sequestration step in rat hepatocytes (Blommaart et al., 1997; Petiot et al., 2000). In order to investigate whether autophagy is involved in MCPIP-mediated OC precursor differentiation, the effect of LY294002 and 3'-MA on expression of autophagy marker Beclin-1, lipidation of LC3 and expression of OC-related genes, TRAP and CTSK was tested. Real-time PCR analysis showed that 3'-MA and LY294002 significantly inhibited MCPIP-induced expression of Beclin-1, TRAP, and CTSK (FIG. **5**D). Immunoblot assay demonstrated that 3'-MA and LY294002 inhibited the expression of Beclin-1, TRAP, and CTSK and lipidation of LC-3 (FIGS. **5**B and C and Supplementary Figure S**4**B and C). However, no effect on expression of GRP78 induced by MCPIP was found revealing that inhibition of autophagy does not affect ER stress that is proposed to cause autophagy (FIGS. **5**B and C and FIGS. **10**B and C). Furthermore, upon inhibition of autophagy by knockdown of Beclin-1 with specific siRNA, MCPIP-induced expression of OC-related markers TRAP and CTSK was markedly suppressed, but scrambled siRNA showed little effects (FIG. **5**E and FIG. **10**D). The chemical inhibitors of autophagy and knockdown of Beclin-1 did not affect MCPIP-induced expression of GRP78, a marker of ER stress, which further leads to autophagy. These results strongly suggested that OC precursor cell differentiation induced by MCPIP expression is mediated via induction of ROS production that causes ER stress, which further leads to autophagy.

To further confirm the involvement of autophagy in OC precursor differentiation, a selective inhibitor of PI3K, wortmannin, was tested (Blommaart et al., 2007) on MCPIP-induced differentiation. Wortmannin severely inhibited MCPIP-induced OC precursor differentiation as indicated by the expression of OC markers at mRNA level by quantitative real-time PCR (qRT-PCR) and protein level by immunoblot analysis (FIG. **6**A-C); inhibition of autophagy was reflected by changes in the Beclin-1 levels. In support of the involvement of autophagy in OC precursor differentiation, knockdown of Atg7 by specific siRNA severely inhibited expression of the OC markers TRAP and CTSK both at mRNA and protein level as measured by qRT-PCR and immunoblot analysis, respectively (FIGS. **11**B and C), whereas scrambled siRNA did not significantly affect induction of these proteins.

Discussion for Examples 1-5:

The role of MCP-1 in differentiation of human bone marrow monocytes to OC precursors has been discovered for the first time herein. Furthermore, it has also been discovered herein that this process is mediated via the induction of MCPIP. MCP-1 induces the differentiation of monocytic cells into TRAP and CTSK-positive cells that do not express other OC functional markers such as αV integrin, β3 integrin, and MMP9 and do not exhibit bone resorption (Kim et al., 2006b). The differentiation into functional OCs requires RANKL in addition to MCP-1. Thus, the MCP-1 induced differentiation yields what might be considered osteoclastogenic cells or OC precursors.

It has been demonstrated that MCPIP mediates MCP-1-induced adipogenesis (Younce et al., 2009), glial differentiation of neuroprogenitor cells (Vrotsos et al., 2009), and angiogenesis (Niu et al., 2008). Here, it was found that forced expression of MCPIP resulted in high expression NADPH oxidase subunit $p47^{PHOX}$ and an increased level of membrane-associated $p47^{PHOX}$, causing ROS production. It is discovered herein that this oxidative stress causes ER stress that leads to autophagy involved in OC differentiation. The involvement of this sequence of processes in OC precursor differentiation is supported by the finding that inhibition of $p47^{PHOX}$ expression, NADPH oxidase activity, ROS production, ER stress, or autophagy by chemical inhibitors or by gene knockdown markedly suppressed MCPIP-induced expression of OC-related genes, TRAP and CTSK.

ROS are associated with multiple cellular functions such as cell proliferation, differentiation, and apoptosis (Wolf, 2005). Many reports revealed that high level of intracellular ROS also contributes to angiogenesis (Xia et al., 2007), epithelial-mesenchymal transition (Zhang et al., 2009), survival, and differentiation of OCs (Steinbeck et al., 1998; Yamasaki et al., 2009). The present results demonstrate that MCP-1 treatment and forced expression of MCPIP induce ROS generation during MCPIP-induced OC precursor differentiation. It has been reported that CeO_2 nanoparticles function as a free radical scavenger (Niu et al., 2007; Tsai et al., 2007; Younce and Kolattukudy, 2010). It has also been identified herein that CeO_2 inhibits the MCP-1- or MCPIP-induced ROS production and expression of OC-related genes.

NADPH oxidase is considered the most important source of ROS by respiratory burst in a monocyte/macrophage system (Decoursey and Ligeti, 2005; Bedard and Krause, 2007). NAD(P)H oxidase is a multiple subunit enzyme complex. Assembly of transmembrane subunits and cytosolic subunits of enzyme complex is the first important step for its activation. In this step, $p47^{PHOX}$ is the most important component which is phosphorylated, translocated from cytoplasm to the membrane to interact with $gp91^{phox}$ (Decoursey and Ligeti, 2005; Bedard and Krause 2007; Leto et al., 2009). Herein, it has been found that MCP-1 treatment or forced expression of MCPIP resulted in expression of $p47^{PHOX}$ and increased the membrane-associated $p47^{PHOX}$ level, and knockdown of MCPIP in MCP-1-treated cells decreased the expression and translocation of $p47^{PHOX}$. Moreover, NADPH oxidase activity inhibitor apocynin and knockdown of $p47^{PHOX}$ with antisense oligonucleotides inhibited membrane translocation of $p47^{PHOX}$, ROS production, and expression of OC-related genes CTSK and TRAP. Thus, MCPIP increases ROS production and induces expression of OC-related genes by increasing expression and translocation of $p47^{PHOX}$. As a zinc-finger protein with a nuclear localization sequence (Zhou et al., 2006) and RNase activity (Matsushita et al., 2009; Skalniak et al., 2009), MCPIP may serve as a novel regulator for several genes at the transcriptional and post-transcriptional level because of its DNA and RNA binding property. Therefore, MCPIP may regulate directly the expression of $p47^{PHOX}$ as a transcriptional factor. Secondly, it has been reported that MCPIP activates MAPK signal pathway (Younce and Kolattukudy, 2010). Activation of MAPK may be an important reason for the MCPIP-induced expression and translocation of $p47^{PHOX}$ oxidase.

ER stress results from the accumulation of misfolded proteins which leads to the induction of the unfolded protein response (UPR) (Malhotra and Kaufman, 2007). ROS production is known to cause proteins to aggregate and misfold. Here, it has been demonstrated that MCP-1 treatment and forced expression of MCPIP induced ER stress via generation of ROS in the monocytes during induction of OC differentiation. The important role of ER stress in monocyte differentiation into OC precursors was demonstrated by the findings that inhibition of ER stress inhibited differentiation and known ER stress inducers caused differentiation. ER stress inhibitor TUDC and knockdown of IRE-1 showed that inhibition of ER stress leads to inhibition of MCPIP-induced OC precursor differentiation. It has also been demonstrated herein that thapsgargin and tunicamycin that are known important inducers of UPR/ER stress induce differentiation of monocytes into OC precursors without MCP-1 or any other inducers. Thapsgargin, an inhibitor of ER-specific Ca-ATPase, has previously been shown to induce OC differentiation from RAW264.7 macrophage cells and mouse bone marrow cells (Takami et al., 1997; Yip et al., 2005).

Autophagy is generally thought of as a survival mechanism, although its dysregulation has been linked to non-apoptotic cell death (Wang, 2008; Glick et al., 2010). Since differentiation involves disappearance of one set of proteins and appearance of a new set of proteins, a self-digestion process such as autophagy could be involved in this process. In fact, autophagy has been reported to be an important event for differentiation of the chronic myelogenous leukemia K562 cells (Colosetti et al., 2009), adipocytes (Malhotra and Kaufman, 2007; Singh et al., 2009; Goldman et al., 2010), paneth cells (Stappenbeck, 2010), and neuronal differentiation (Zeng and Zhou, 2008). Beclin-1 is a critical component in the class III PI3K complex (PI3KC3) that induces the formation of autophagosomes in mammalian systems (Wang, 2008). It has been demonstrated that Beclin-1 bridges autophagy and differentiation, and the process of autophagy and differentiation requires up-regulation of Beclin-1 (Wang, 2008). Inhibition of differentiation by PI3K inhibitors and knockdown of Beclin-1 and Atg7 strongly suggest that MCPIP induced OC precursor differentiation via autophagy. Emerging data now indicate that ER stress is a potent inducer of autophagy (Sakaki and Kaufman, 2008). Herein, the inventors have discovered that MCPIP induces OC precursor differentiation via autophagy which depends on MCPIP-induced ROS production and ER stress. It has been reported that ER stress can activate p38 MAPK signal pathway to induce autophagy (Kim et al., 2008). Moreover, ER stress activates phosphorylation of protein kinase-like ERK (PERK), an ER-localized transmembrane protein, and induction of IRE-1 is necessary for radiation-induced autophagy in mouse embryonic fibroblasts (Kim et al., 2010). Activation of NF-KB and MAPK signal pathway is necessary for OC differentiation (Huang et al., 2006). However, it was reported that MCPIP inhibits activation of NF-KB induced by IL-16 (Skalniak et al., 2009) and lipopolysaccharide stimulation (Liang et al., 2008) while over-expression of MCPIP can cause activation of JNK and p38 (Younce and Kolattukudy, 2010). Thus, activation of p38 instead of NF-κB signaling is probably involved in MCPIP-mediated differentiation of monocytes into OC precursors. The overall pathway involved in the MCP-1/MCPIP-mediated OC precursor differentiation is shown in FIG. **12**.

Inflammatory bone erosion is involved in many pathological conditions (Lu et al., 2007; Ha et al., 2010). The novel inventive features recited herein provide a new insight into the mechanism by which MCP-1 induces differentiation of monocytic cells into TRAP- and CTSK-expressing cells that can proceed to differentiate into functional OCs in the presence of RANKL, and suggest that MCPIP is a novel target for therapy of inflammatory bone erosion.

Materials and Methods Related to Examples 1-5:

Reagents and Antibodies

Human BMCs were from Stemcell Technologies. α-MEM, FBS, HBSS, trypsin, recombinant human M-CSF (300-25), human MCP-1, and Trizol reagent were purchased from Invitrogen. Anti-β-actin, CTSK monoclonal antibodies, CeO_2 nanoparticles, apocynin, TUDC, 3'-MA, and LY294002 were from Sigma-Aldrich. Anti-TRAP, $p47^{PHOX}$, Fas, IRE-1, GRP78, Beclin-1, LC3 polyclonal antibodies, goat anti-rabbit and mouse secondary antibodies, and specific siRNA for IRE-1 and Beclin-1 were purchased from Santa Cruz Biotechnology. Specific siRNA for MCPIP and negative control siRNA were obtained from Ambion. Anti-MCPIP polyclonal antibody was prepared as indicated before (Zhou et al., 2006; Younce and Kolattukudy, 2010).

Cell Culture and Treatment

BMCs were cultured in α-MEM supplemented with 10% FBS containing 30 ng/ml M-CSF, 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2 at 37° C. OC precursor cells were induced after 3-day culture as Ha et al. (2010). At this point, cells were treated with 50 ng/ml MCP-1, inhibitors, or gene transfection. For inhibitor treatment, CeO_2 (10 μM), apocynin (100 μg/ml), TUDC (100 μM), 3'-MA (50 μM), and LY294002 (20 μM) were added 6 h before gene transfection.

Gene Transfection and siRNA Knockdown

OC precursor cells were transfected with 1 μg GFP or MCPIP-GFP eukaryotic expression plasmids for gene-gain-function assay by using Fugene 6. For gene silencing, chemically synthesized siRNA duplex (100 nM) targeting MCPIP, IRE-1, or Beclin-1 was transfected into OC precursor cells using DharmaFECT (Dharmacon) for 24 h prior to transfection with MCPIP-GFP or GFP plasmid. A scrambled siRNA was used as a negative control. For knockdown expression of $p47^{PHOX}$, specific antisense (AS) (5'-CCAGCAGGGCGATGTGACGGATGAA-3' (SEQ ID NO: 5)) and sense (5'-ATGGGGGACACCTTCATCCGTCAC-3' (SEQ ID NO: 6)) oligonucleotides were designed and synthesized by phosphorothioate modification by Integrated DNA Technologies. The oligonucleotides were transfected into OC precursor cells using Lipofectamine 2000 for 24 h before MCPIP-GFP plasmid transfection.

TRAP Staining

Three days after MCP-1 treatment or 4 days after MCPIP transfection, cells were fixed for histological staining for TRAP as described previously (Kim et al., 2006a). Briefly, following fixation, cells were stained with freshly prepared TRAP staining solution (naphthol AS-MX phosphate, fast red violet LB salt, and potassium sodium tartrate). On each coverslip, totally at least 500 cells were examined and the TRAP-positive cells were counted in 3-5 fields (20× objective), and the percentage of TRAP-positive cells was calculated.

Quantitative Real-Time Polymerase Chain Reaction

Cells were centrifuged after wash with PBS for twice. Cell pellet was resuspended in 1 ml Trizol reagent and the total RNA was extracted with chloroform and isopropanol, purified on Qiagen Mini-Prep column, and treated with DNase. High Capacity cDNA Reverse Transcription kit (Applied Biosystems) was used for cDNA preparation from 1 μg total RNA. qRT-PCR was done in triplicate in an ABI PRISM 7900HT Sequence Detection System with 5%

cDNA product, primers (Supplementary Table S1) at 125 nM, and Fast SYBR Green Master Mix (Applied Biosystems). Relative quantitation of PCR products was done by the $2^{-\Delta\Delta CT}$ method, CT=cycles to threshold, and $^{\Delta\Delta}$CT= (target gene CT)−(β-actin reference gene CT). Final data were described as fold changes against control cells.

ROS Production

Oxidant production in OC precursor cells was assessed by measuring the oxidation of intracellular DHR123 (Molecular Probes) as described previously (Younce et al., 2010). ROS production was expressed as folds compared with control cells expressing GFP alone.

Membrane Isolation

OC precursor cells were harvested, sonicated on ice in a buffer containing 100 mM KCl, 3 mM NaCl, 3.5 mM $MgCl_2$, 10 mM HEPES, 1 mM EGTA, 10 μg/ml pepstatin, 10 μg/ml leupeptin, and 0.5 mM PMSF; lysates were centrifuged at 600 g for 10 min at 4° C. to remove nuclei and unbroken cells. The supernatant was then ultracentrifuged at 100000 g for 1 h at 4° C. Membranes were washed in the same buffer, quantified (Lemarie), and resuspended in Laemmli sample buffer, before western blot analysis.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Western Blotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting were performed as described elsewhere. Samples (30 μg of protein) were subjected to 12% SDS-PAGE for most of target protein and 18% SDS-PAGE for LC3-II and LC3, transferred onto PVDF membranes (Millipore), and assayed for MCPIP, $p47^{PHOX}$, TRAP, CTSK, GRP78, IRE-1, Beclin-1, LC3-II, and β-actin or Fas (loading control) protein expression by chemiluminescence detection (Pierce ECL kit) according to the manufacturer's instructions. The specific protein bands were quantified by densitometric analysis with GS-690 Image Densitometer (Bio-Rad).

Statistical Analysis

Data are represented as mean±SD of experiments performed on at least three separate occasions. Student's t-test was used to compare the means of normally distributed continuous variables. $P<0.05$ indicated statistical significance.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of molecular biology. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Reference is made to standard textbooks of molecular biology that contain definitions and methods and means for carrying out basic techniques, encompassed by the present invention. See, for example, Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1982) and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1989); Methods in Plant Molecular Biology, Maliga et al, Eds., Cold Spring Harbor Laboratory Press, New York (1995); Arabidopsis, Meyerowitz et al, Eds., Cold Spring Harbor Laboratory Press, New York (1994) and the various references cited therein.

Finally, while various embodiments of the present invention have been shown and described herein, it will be obvious that such embodiments are provided by way of example only. Numerous variations, changes and substitutions may be made without departing from the invention herein. Accordingly, it is intended that the invention be limited only by the spirit and scope of the appended claims. The teachings of all patents and other references cited herein are incorporated herein by reference in their entirety to the extent they are not inconsistent with the teachings herein.

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Supplementary Table S1 Primers for human genes tested in this study.

Product	Primer sequence	
MCPIP	sense	5'-GTTTCCAACGACACATACCGTGAC-3' (SEQ ID NO: 7)
	antisense	5'-CTTCTTACG CAGGAAGTTGTCCAG-3' (SEQ ID NO: 8)
TRAP	sense	5'-GCAGATCCTGGGTGCAGACTTC-3' (SEQ ID NO: 9)
	antisense	5'-GGGAGCGGTCAGAGAATACGTGC-3' (SEQ ID NO: 10)
CTSK	sense	5'-GAGGGGGCTACATGACCAATGC-3' (SEQ ID NO: 11)
	antisense	5'-CTGCCTTGCCTGTTGGGTTGA-3' (SEQ ID NO: 12)
GRP78	sense	5'-ACAGCTTCTGATAATCAACCAA-3' (SEQ ID NO: 13)
	antisense	5'-ACTTCAATCTGTGGGACCC-3' (SEQ ID NO: 14)
IRE-1	sense	5'-ACACCATCACCATGTACGACACCA-3' (SEQ ID NO: 15)
	antisense	5'-ATTCAC TGTCCACAGTCACCACCA-3' (SEQ ID NO: 16)
IRE-1	sense	5'-ACACCATCACCATGTACGACACCA-3' (SEQ ID NO: 15)
	antisense	5'-ATTCAC TGTCCACAGTCACCACCA-3' (SEQ ID NO: 16)
MMP9	sense	5'-TACCACCTCGAACTTTGACAGCGA-3' (SEQ ID NO: 17)
	antisense	5'-GCCATTCACGTCGTCCTTATGCAA-3' (SEQ ID NO: 18)
αV integrin	sense	5'-T TCCAAACTGGGAGCACAAGGAGA-3' (SEQ ID NO: 19)
	antisense	5'-TGTAAGGCCACTGAAGATG GAGCA-3' (SEQ ID NO: 20)
β3 integrin	sense	5'-CTCCTGTGTCCGCTACAAGGG-3' (SEQ ID NO: 21)
	antisense	5'-GTCCAGTCGGAGTCACACAGG-3' (SEQ ID NO: 22)
beclin-1	sense	5'-CCGTGTCACCATCCAGGAACTC-3' (SEQ ID NO: 23)
	antisense	5'-ACCATCCTGGCGAGGAGTTTC-3' (SEQ ID NO: 24)
Atg7	sense	5'-ATGTGGTGGCCCCAGGAGAT3' (SEQ ID NO: 25)
	antisense	5'-AGATACCATCAATTCCACGG-3' (SEQ ID NO: 26)
β-actin	sense	5'-GAGGCACTCTTCCAGCCTTCC-3' (SEQ ID NO: 27)
	antisense	5'-GCGGATGTCCACGTCACACTT-3' (SEQ ID NO: 38)

Scheme 1

MCP-1

↓

MCPIP

↓

NADPH oxidase

↓

Oxidative stress

↓

ER Stress

↓

-continued

Autophagy

↓

Osteoclast Precursor Differentiation

SEQUENCE LISTING

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gaggaggcct cggccctgga actgcagatg aaggtggact tcttccggaa gctgggctat 180

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gtgctgggtg agctggtgaa acacgggaca gccaccgagc gggagcgcca gacctcaccg 300

gacccctgcc ctcagctccc tctagtcccg cggggtggtg gcacccctaa ggctcccaac 360

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tgtggtgtgt ttcccccgca cctggtggag gctgtgatgg ggcgcttccc acagctcctg 1740

gacccccagc agctggctgc cgagatcctc tcctacaagt cccagcaccc cagtgagtaa 1800

<210> SEQ ID NO 2
<211> LENGTH: 599
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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1               5                  10                  15

Met Ser Leu Trp Glu Phe Glu Asp Ser His Ser Arg Gln Gly Thr Pro
            20                  25                  30

Arg Pro Gly Gln Glu Leu Ala Ala Glu Glu Ala Ser Ala Leu Glu Leu
        35                  40                  45

Gln Met Lys Val Asp Phe Phe Arg Lys Leu Gly Tyr Ser Ser Thr Glu
    50                  55                  60

Ile His Ser Val Leu Gln Lys Leu Gly Val Gln Ala Asp Thr Asn Thr
65                  70                  75                  80

Val Leu Gly Glu Leu Val Lys His Gly Thr Ala Thr Glu Arg Glu Arg
                85                  90                  95

Gln Thr Ser Pro Asp Pro Cys Pro Gln Leu Pro Leu Val Pro Arg Gly
            100                 105                 110

Gly Gly Thr Pro Lys Ala Pro Asn Leu Glu Pro Pro Leu Pro Glu Glu
        115                 120                 125

Glu Lys Glu Gly Ser Asp Leu Arg Pro Val Val Ile Asp Gly Ser Asn
    130                 135                 140

Val Ala Met Ser His Gly Asn Lys Glu Val Phe Ser Cys Arg Gly Ile
145                 150                 155                 160

Leu Leu Ala Val Asn Trp Phe Leu Glu Arg Gly His Thr Asp Ile Thr
                165                 170                 175

Val Phe Val Pro Ser Trp Arg Lys Glu Gln Pro Arg Pro Asp Val Pro
            180                 185                 190

Ile Thr Asp Gln His Ile Leu Arg Glu Leu Glu Lys Lys Lys Ile Leu
        195                 200                 205

Val Phe Thr Pro Ser Arg Arg Val Gly Gly Lys Arg Val Val Cys Tyr
    210                 215                 220

Asp Asp Arg Phe Ile Val Lys Leu Ala Tyr Glu Ser Asp Gly Ile Val
225                 230                 235                 240

Val Ser Asn Asp Thr Tyr Arg Asp Leu Gln Gly Glu Arg Gln Glu Trp
                245                 250                 255

Lys Arg Phe Ile Glu Glu Arg Leu Leu Met Tyr Ser Phe Val Asn Asp
            260                 265                 270

Lys Phe Met Pro Pro Asp Asp Pro Leu Gly Arg His Gly Pro Ser Leu
        275                 280                 285

Asp Asn Phe Leu Arg Lys Lys Pro Leu Thr Leu Glu His Arg Lys Gln
    290                 295                 300

Pro Cys Pro Tyr Gly Arg Lys Cys Thr Tyr Gly Ile Lys Cys Arg Phe
305                 310                 315                 320

Phe His Pro Glu Arg Pro Ser Cys Pro Gln Arg Ser Val Ala Asp Glu
                325                 330                 335

Leu Arg Ala Asn Ala Leu Leu Ser Pro Pro Arg Ala Pro Ser Lys Asp
            340                 345                 350

Lys Asn Gly Arg Arg Pro Ser Pro Ser Ser Gln Ser Ser Ser Leu Leu
        355                 360                 365

Thr Glu Ser Glu Gln Cys Ser Leu Asp Gly Lys Lys Leu Gly Ala Gln
    370                 375                 380

Ala Ser Pro Gly Ser Arg Gln Glu Gly Leu Thr Gln Thr Tyr Ala Pro
385                 390                 395                 400

Ser Gly Arg Ser Leu Ala Pro Ser Gly Gly Ser Gly Ser Ser Phe Gly
                405                 410                 415
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Pro Thr Asp Trp Leu Pro Gln Thr Leu Asp Ser Leu Pro Tyr Val Ser
            420                 425                 430

Gln Asp Cys Leu Asp Ser Gly Ile Gly Ser Leu Glu Ser Gln Met Ser
        435                 440                 445

Glu Leu Trp Gly Val Arg Gly Gly Gly Pro Gly Glu Pro Gly Pro Pro
    450                 455                 460

Arg Ala Pro Tyr Thr Gly Tyr Ser Pro Tyr Gly Ser Glu Leu Pro Ala
465                 470                 475                 480

Thr Ala Ala Phe Ser Ala Phe Gly Arg Ala Met Gly Ala Gly His Phe
                485                 490                 495

Ser Val Pro Ala Asp Tyr Pro Pro Ala Pro Pro Ala Phe Pro Pro Arg
            500                 505                 510

Glu Tyr Trp Ser Glu Pro Tyr Pro Leu Pro Pro Pro Thr Ser Val Leu
        515                 520                 525

Gln Glu Pro Pro Val Gln Ser Pro Gly Ala Gly Arg Ser Pro Trp Gly
    530                 535                 540

Arg Ala Gly Ser Leu Ala Lys Glu Gln Ala Ser Val Tyr Thr Lys Leu
545                 550                 555                 560

Cys Gly Val Phe Pro Pro His Leu Val Glu Ala Val Met Gly Arg Phe
                565                 570                 575

Pro Gln Leu Leu Asp Pro Gln Gln Leu Ala Ala Glu Ile Leu Ser Tyr
            580                 585                 590

Lys Ser Gln His Pro Ser Glu
        595
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<210> SEQ ID NO 3
<211> LENGTH: 1791
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 3

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agtcttgagg acagacacag cagccagggt cgacctcagc cagaccagga tcctgtggct    120

aaagaggccc ctacttccga gctgcagatg aaggtggact ttttccgtaa actggggtac    180

tcgtcctctg agatccacag tgtcctgcag aagctgggag tccaagcaga caccaacacg    240

gtgctagggg aattggtgaa gcatggctca gctactgaac gagaatgcca ggccctgacg    300

gcccccagcc cccagccccc tctggtgccc cggggtggaa gcacccccaa gccttccact    360

ctagaaccct cactcccaga ggaggacaga gagggcagcg acctgagacc tgtggtcatc    420

gacggaagca atgtggccat gagccatggg aacaaggaag tcttctcttg ccggggcatt    480

ctgctggctg tgaactggtt tctggagcga ggccacacag atattaccgt gtttgtgcca    540

tcttggagga aggaacagcc tcgaccagat gtgcctatca cagaccagca catccttcgg    600

gaactagaga aaaagaagat cttggtgttc acgccatcca ggcgggtcgg cggcaagcgc    660

gtggtgtgct atgatgaccg cttcattgtg aagctggcct tcgaatccga cggagtggtg    720

gtctccaatg acacgtaccg agacctccaa ggcgagaggc aggagtggaa acgcttcatc    780

gaggagcggc tgctcatgta ctccttcgtc aatgacaagt tcatgccccc tgacgaccct    840

ttaggacggc atgggcctag cctggacaac ttccttcgta agaaaccact gccttctgag    900

cacaggaagc agccatgccc ctatgggaag aaatgtacgt atgaaatcaa gtgccgattt    960

ttccaccctg agcggccaag ccgtccccag cgctctgtgg ccgatgagct ccgtgccaac   1020

gctctcctct cacctcccag gactccagtc aaggacaaaa gtagccagag gccttccccct  1080
```

-continued

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gcctctcagt ccagctctgt gtccctagag gctgaaccag gcagcctgga tgggaaaaag   1140

ctgggtgcca ggtcatctcc gggtccccac cgagaaggct caccgcagac ctgtgctcca   1200

gctggcagga gcctccctgt tagtgggggc agctttgggc ccacagagtg gcttgcacac   1260

acccaggact cactcccata cacctcccag gagtgccttg attcaggcat tggttccctg   1320

gagagccaga tgtcagaatt atggggcgtg cgaggaggca gccctgggga gtcgggcccc   1380

actcggggcc cctatgcagg ttatcacagc tatggatcca aggtcccagc agcaccttcc   1440

ttttctcctt ttagaccagc catgggtgct ggccacttca gtgtccccac cgactatgtg   1500

cccccgccac ccacctaccc atccagagag tactggtctg agccgtaccc attaccccca   1560

cccactcctg tccttcagga gccccagaga cccagccccg gggctggtgg gggcccctgg   1620

ggcagggtgg gtgacctggc caaagaaagg gctggtgtat ataccaagct gtgtggtgtc   1680

ttccccccac acctggtaga agctgtaatg agacgcttcc cacagctgct ggatccgcag   1740

cagctggccg cagagatcct gtcttacaag tcccagcacc tcagtgagta a            1791
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<210> SEQ ID NO 4
<211> LENGTH: 596
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

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Met Ser Asp Pro Cys Gly Thr Lys Pro Val Gln Glu Ser Asn Pro Thr
1               5                   10                  15

Met Ser Leu Trp Ser Leu Glu Asp Arg His Ser Ser Gln Gly Arg Pro
            20                  25                  30

Gln Pro Asp Gln Asp Pro Val Ala Lys Glu Ala Pro Thr Ser Glu Leu
        35                  40                  45

Gln Met Lys Val Asp Phe Phe Arg Lys Leu Gly Tyr Ser Ser Ser Glu
    50                  55                  60

Ile His Ser Val Leu Gln Lys Leu Gly Val Gln Ala Asp Thr Asn Thr
65                  70                  75                  80

Val Leu Gly Glu Leu Val Lys His Gly Ser Ala Thr Glu Arg Glu Cys
                85                  90                  95

Gln Ala Leu Thr Ala Pro Ser Pro Gln Pro Pro Leu Val Pro Arg Gly
            100                 105                 110

Gly Ser Thr Pro Lys Pro Ser Thr Leu Glu Pro Ser Leu Pro Glu Glu
        115                 120                 125

Asp Arg Glu Gly Ser Asp Leu Arg Pro Val Val Ile Asp Gly Ser Asn
    130                 135                 140

Val Ala Met Ser His Gly Asn Lys Glu Val Phe Ser Cys Arg Gly Ile
145                 150                 155                 160

Leu Leu Ala Val Asn Trp Phe Leu Glu Arg Gly His Thr Asp Ile Thr
                165                 170                 175

Val Phe Val Pro Ser Trp Arg Lys Glu Gln Pro Arg Pro Asp Val Pro
            180                 185                 190

Ile Thr Asp Gln His Ile Leu Arg Glu Leu Glu Lys Lys Lys Ile Leu
        195                 200                 205

Val Phe Thr Pro Ser Arg Arg Val Gly Gly Lys Arg Val Val Cys Tyr
    210                 215                 220

Asp Asp Arg Phe Ile Val Lys Leu Ala Phe Glu Ser Asp Gly Val Val
225                 230                 235                 240
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Val Ser Asn Asp Thr Tyr Arg Asp Leu Gln Gly Glu Arg Gln Glu Trp
                245                 250                 255

Lys Arg Phe Ile Glu Glu Arg Leu Leu Met Tyr Ser Phe Val Asn Asp
            260                 265                 270

Lys Phe Met Pro Pro Asp Asp Pro Leu Gly Arg His Gly Pro Ser Leu
        275                 280                 285

Asp Asn Phe Leu Arg Lys Lys Pro Leu Pro Ser Glu His Arg Lys Gln
    290                 295                 300

Pro Cys Pro Tyr Gly Lys Lys Cys Thr Tyr Glu Ile Lys Cys Arg Phe
305                 310                 315                 320

Phe His Pro Glu Arg Pro Ser Arg Pro Gln Arg Ser Val Ala Asp Glu
                325                 330                 335

Leu Arg Ala Asn Ala Leu Leu Ser Pro Pro Arg Thr Pro Val Lys Asp
            340                 345                 350

Lys Ser Ser Gln Arg Pro Ser Pro Ala Ser Gln Ser Ser Ser Val Ser
        355                 360                 365

Leu Glu Ala Glu Pro Gly Ser Leu Asp Gly Lys Lys Leu Gly Ala Arg
    370                 375                 380

Ser Ser Pro Gly Pro His Arg Glu Gly Ser Pro Gln Thr Cys Ala Pro
385                 390                 395                 400

Ala Gly Arg Ser Leu Pro Val Ser Gly Gly Ser Phe Gly Pro Thr Glu
                405                 410                 415

Trp Leu Ala His Thr Gln Asp Ser Leu Pro Tyr Thr Ser Gln Glu Cys
            420                 425                 430

Leu Asp Ser Gly Ile Gly Ser Leu Glu Ser Gln Met Ser Glu Leu Trp
        435                 440                 445

Gly Val Arg Gly Gly Ser Pro Gly Glu Ser Gly Pro Thr Arg Gly Pro
    450                 455                 460

Tyr Ala Gly Tyr His Ser Tyr Gly Ser Lys Val Pro Ala Ala Pro Ser
465                 470                 475                 480

Phe Ser Pro Phe Arg Pro Ala Met Gly Ala Gly His Phe Ser Val Pro
                485                 490                 495

Thr Asp Tyr Val Pro Pro Pro Pro Thr Tyr Pro Ser Arg Glu Tyr Trp
            500                 505                 510

Ser Glu Pro Tyr Pro Leu Pro Pro Pro Thr Pro Val Leu Gln Glu Pro
        515                 520                 525

Gln Arg Pro Ser Pro Gly Ala Gly Gly Gly Pro Trp Gly Arg Val Gly
    530                 535                 540

Asp Leu Ala Lys Glu Arg Ala Gly Val Tyr Thr Lys Leu Cys Gly Val
545                 550                 555                 560

Phe Pro Pro His Leu Val Glu Ala Val Met Arg Arg Phe Pro Gln Leu
                565                 570                 575

Leu Asp Pro Gln Gln Leu Ala Ala Glu Ile Leu Ser Tyr Lys Ser Gln
            580                 585                 590

His Leu Ser Glu
        595


<210> SEQ ID NO 5
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 5
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<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 6

atgggggaca ccttcatccg tcac 24

<210> SEQ ID NO 7
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 7

gtttccaacg acacataccg tgac 24

<210> SEQ ID NO 8
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 8

cttcttacgc aggaagttgt ccag 24

<210> SEQ ID NO 9
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 9

gcagatcctg ggtgcagact tc 22

<210> SEQ ID NO 10
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 10

gggagcggtc agagaatacg tgc 23

<210> SEQ ID NO 11
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 11

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gaggggggcta catgaccaat gc 22

<210> SEQ ID NO 12
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 12

ctgccttgcc tgttgggttg a 21

<210> SEQ ID NO 13
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 13

acagcttctg ataatcaacc aa 22

<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 14

acttcaatct gtgggaccc 19

<210> SEQ ID NO 15
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 15

acaccatcac catgtacgac acca 24

<210> SEQ ID NO 16
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 16

attcactgtc cacagtcacc acca 24

<210> SEQ ID NO 17
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 17

taccacctcg aactttgaca gcga 24

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<210> SEQ ID NO 18
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 18

gccattcacg tcgtccttat gcaa 24

<210> SEQ ID NO 19
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 19

ttccaaactg ggagcacaag gaga 24

<210> SEQ ID NO 20
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 20

tgtaaggcca ctgaagatgg agca 24

<210> SEQ ID NO 21
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 21

ctcctgtgtc cgctacaagg g 21

<210> SEQ ID NO 22
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 22

gtccagtcgg agtcacacag g 21

<210> SEQ ID NO 23
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 23

ccgtgtcacc atccaggaac tc 22

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<210> SEQ ID NO 24
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 24

accatcctgg cgaggagttt c 21

<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 25

atgtggtggc cccaggagat 20

<210> SEQ ID NO 26
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 26

agataccatc aattccacgg 20

<210> SEQ ID NO 27
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 27

gaggcactct tccagccttc c 21

<210> SEQ ID NO 28
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 28

gcggatgtcc acgtcacact t 21

What is claimed is:

1. A method of treating a condition in a patient in need, the method comprising administering to the patient a therapeutically effective amount of a composition that inhibits the expression or action of MCPIP, wherein said condition comprises abnormal osteoclast-induced bone resorption, wherein the composition comprises siRNA specific for MCPIP, shRNA specific for MCPIP, or antisense nucleotide specific to an mRNA sequence encoding MCPIP.

2. The method of claim **1**, wherein the patient in need is exhibiting pre-arthritic symptoms.

3. The method of claim **1**, wherein the patient in need is exhibiting pre-osteoporotic symptoms.

4. The method of claim **1** wherein the composition comprises siRNA specific for MCPIP.

5. The method of claim **1** wherein the composition comprises an antisense nucleotide specific for MCPIP.

6. The method of claim **1** wherein the composition comprises shRNA specific for MCPIP.

7. The method of claim **1**, wherein the composition is administered to the patient via intrabuccal, oral, rectal, pulmonary, ocular, or transdermal administration.

8. The method of claim 1, wherein the condition comprises an osteoporosis-related condition.

9. The method of claim 1, wherein the condition comprises Rheumatoid Arthritis.

10. The method of claim 1, wherein inhibiting MCPIP levels comprises directly administering the composition to the patient in need.

11. The method of claim 1, wherein administering the composition comprises administering a composition comprising a therapeutically effective amount of the composition that inhibits the expression or action of MCPIP and a pharmaceutically acceptable excipient.

12. A method of inhibiting osteoclast production in a patient in need, comprising:

administering a therapeutically effective amount of a composition that inhibits the expression or action of MCPIP in the patient, wherein the composition comprises siRNA specific for MCPIP, shRNA specific for MCPIP, or an antisense nucleotide specific to an mRNA sequence encoding MCPIP.

13. The method of claim 12, wherein the patient in need is exhibiting symptoms of rheumatoid arthritis, osteoarthritis, and/or osteoporosis.

14. The method of claim 12, wherein administering a therapeutically effective amount of a composition includes a composition comprising: a composition that inhibits the expression or action of MCPIP, and a pharmaceutically acceptable excipient.

15. The method of claim 12, wherein the composition includes an MCPIP siRNA, an shRNA specific for MCPIP and/or an antisense nucleotide specific for MCPIP.

16. The method of claim 1, wherein the condition is an inflammatory joint disease.

17. A method of treating an inflammatory disease in a patient in need thereof, said method comprising:

procuring human bone marrow mononuclear cells (BMCs) from said patient to obtain procured BMC's;

treating said procured BMC's, ex vivo, by blocking expression of MCPIP in said cells to obtain treated BMC's, wherein the procured BMCs are treated with a composition comprising siRNA specific for MCPIP, shRNA specific for MCPIP, or an antisense nucleotide specific to an mRNA sequence encoding MCPIP;

administering said treated BMC's to said patient.

18. The method of claim 17, wherein said treating comprises subjecting said procured BMC's to an antisense nucleotide specific to an mRNA sequence encoding MCPIP.

19. The method of claim 17, wherein said treating comprises subjecting said procured BMC's to siRNA specific to MCPIP.

20. The method of claim 17, wherein said inflammatory disease comprises osteoporosis, or arthritis.

* * * * *